FINAL REPORT

INTEGRATED HOST MANAGEMENT: THE EFFECT OF DUAL MITE INFESTATION OF A HONEY BEE COLONY

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Integrated Host Management in Honey Bees and Impact of Multiple Mite Infestations in Bee Hives

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Parasitic mites present the greatest economic threat to beekeeping in North America today. There are two species of concern, the honey bee tracheal mite *Acarapis woodi* and the varroa mite *Varroa jacobsoni*. Tracheal mites infest adult bees, living inside the tracheal tubes. Their whole life cycle is contained there, and they only leave the trachea to transfer to the next host. Varroa mites have two life cycle hosts, beginning with a female varroa entering a cell just before it is sealed to feed on the larvae as it pupates. The female and her offspring emerge with the bee and spend some time on adult bees before returning to a cell.

Both mite species can be fatal to colonies, and much work has been done to understand the impact of both mites independently. Varroa mites are known to reduce honey and brood production, increase drift, and increase winter bee and colony mortality. Tracheal mites are known to decrease honey and brood production, increase winter colony mortality and are associated with higher bacterial and viral disease incidence. As the mites continue to spread, beekeepers are routinely experiencing dual infestations in their colonies, but no research has been done to understand this condition. Understanding the impact of dual infestation at the colony level and at the individual bee level is critical to the beekeeping industry in order to devise appropriate management methods.

Our work focussed on three major questions: 1) How does colony mortality vary with single and dual infestations? 2) How do tracheal and varroa mites affect colony production of adult bees, brood, and storage of honey and pollen? and 3) Can management tools be developed to treat for both mites simultaneously?

The most important finding of this project was that dual infestations are considerably worse than infestations of tracheal or varroa mites alone. Beekeepers today are spending considerably more time and money dealing with varroa mites, and our findings indicate that it is important not to neglect tracheal mite management.

The details of our research will be available in scientific journals shortly. Three manuscripts from this project currently are in press, and included with this report. Below is a point-form summary of the principal research results from these studies:

- Proportion of pollen collection was not different from colonies with single or dual infestations, but bees parasitized by both mites had significantly smaller pollen loads than uninfested bees.

- The mean onset of foraging was earliest for bees parasitized by varroa during development (15.9 days), latest for tracheal mite-parasitized bees (20.5 days), and intermediate for control and varroa-infested bees (17.6 and 18.0 respectively).

- Phoretic varroa were found equally on bees with and without tracheal mite infestations, but bees parasitized by varroa during development were almost twice as likely to have tracheal mite infestations as bees with no varroa parasitism.

- Colony mortality was considerably greater in colonies infested with both mite species than with single infestations. Within the first year, 80% of dually-infested colonies were dead, and none of the control or single-infested colonies. Varroa infestation was the second most significant cause of mortality.

- Colony productivity was not measurable in dually-infested colonies, since most died quickly. For the other treatments, the varroa-infested colonies produced poorly, and the control and tracheal mite colonies did well.

- Various neem and botanical oil treatments were tried to control both mites, and summer treatments with oil sprays showed some potential for commercial mite control.

Reporting of Results

We have made considerable effort to present the results of our findings to beekeeping audiences, in addition to our scientific publications. Reports of this project have been published regularly in the Saskatchewan beekeeping newsletter put out by Saskatchewan Agriculture, as well as in *Hive Lights*, the newsletter of the Canadian Honey Council.

In addition, we have traveled extensively to present results at beekeeping meetings, including annual attendance and talks presented at the Saskatchewan Beekeepers Association meeting, as well as the national meeting of the Canadian Honey Council. Further, these results have been presented at local, national, and international meetings. Just this year, I have already spoken in Saskatchewan, Manitoba, Illinois, and England to present these findings to beekeeping audiences. Finally, papers were given at the international Apimondia meeting in September 1999, held in Vancouver, at which 3000 people were in attendance.

In summary, I believe this project accomplished its objectives, and has provided some important findings for bee management. We have been diligent, indeed enthusiastic, about interacting with beekeepers around the world to distribute these results in a beekeeper-friendly fashion, in addition to the more "traditional" route of publishing in scientific journals. We greatly appreciate the support provided by the Agri-Food

Innovation Fund, and in our written and verbal presentations have been pleased to be able to acknowledge that important support.

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Honey Bee Colony Mortality and Productivity with Single and Dual Infestations of Parasitic Mite Species

running head: Two Mites, Honey Bee Productivity & Mortality

D. L. Downey* and M. L. Winston

ABSTRACT

Colony mortality and productivity were compared between honey bee *(Apis mellifera* L) colonies infested by zero, one or both species of parasitic mites (*Acarapis woodi* or *Varroa jacobsoni*). Mortality, bee and mite populations, sealed brood, and stores were monitored for 16 months, beginning in May. By March, 83% of colonies with both mites were dead, but no other colonies died until September, when 75% of varroa colonies were dead. Dually infested colonies initially had more honey stores, but were dead by March. At that point varroa colonies had significantly less worker brood, fewer adult bees and more honey than colonies with no mites or tracheal mites. The colonies with tracheal mites and no mites were not different on any productivity parameters measured. These results indicate a synergistic interaction between tracheal and varroa mites, and suggest that treatments against tracheal mites should be applied in dually infested colonies, even if tracheal mites alone are not having an impact.

Keywords: ***Varroa jacobsoni/ Acarapis woodi/*** **colony productivity/ colony mortality**

INTRODUCTION

The honey bee *Apis mellifera* L. is important for honey production and pollination world-wide, and thus this species has been moved and traded across the globe. Movement of bee stock has facilitated distribution of parasites and pathogens that exploit the homeostatic environment of honey bee colonies, and these have become critical management problems. The varroa mite, *Varroa jacobsoni* Oud. and the honey bee tracheal mite, *Acarapis woodi* are two parasites that are primary concerns for beekeepers. The varroa mite is a highly destructive pest, responsible for reduced honey (Currie pers. com.) and brood (Delfinado-Baker 1988) production, precocious foraging (Schneider 1986), increased drift (Sakofski 1990), higher bee mortality in winter (Kovac and Crailsheim 1988) and considerable colony mortality (De Jong 1997). The original host of this mite is the Asian honey bee, *Apis cerana* Fabr., and as varroa enters *Apis mellifera* populations worldwide, beekeepers experience substantial colony loss and damage.

Tracheal mites are pests of more variable impact. Several British studies of the tracheal mite suggest that it may not be a current threat to honey bee colonies, although it is thought to be responsible for devastating colony losses in the early century, then called the Isle of Wight Disease. In North America, especially in colder climates, studies indicate that tracheal mites are a more serious pest. They can reduce honey yield (Eischen et. al 1989) and brood

production (Eischen 1987), increase winter mortality (Otis et al. 1988, 1992) and are associated with higher bacterial and viral disease incidence (Bailey & Ball 1991). Varroa mites are consistently more virulent than tracheal mites, often killing a colony within two years if left untreated (Bailey & Ball 1991). This, combined with variable tracheal mite impact and sampling methods requiring a microscope, results in many beekeepers focusing management attention on varroa and neglecting tracheal mite sampling and treatment.

Varroa mites are relatively large ectoparasites that develop and reproduce in the cell of a developing bee. Adult females enter the cell just before it is capped for pupation, lay eggs and puncture the bee to feed on its hemolymph. The mite and its offspring usually do not kill the bee, they emerge with it to undergo a phoretic period on adult bees before they enter another cell to reproduce. The tracheal mite carries out its whole life cycle in the trachea of adult honey bees, puncturing the trachea and feeding on hemolymph, reproducing as long as the bee lives (Pettis and Wilson, 1996). Tracheal mite progeny leave the trachea only to find another susceptible host. Mite studies to date have investigated varroa and tracheal mites independently, but as they continue to spread, it is common to have both mites in one colony.

The objective of this project was to assess the impact of dual infestation on honey bee colonies. Since both mites feed on bee hemolymph and can have some similar effects on colonies independently, we predicted that dual infestation

would have an additive or synergistic effect on colony productivity and mortality.

MATERIALS AND METHODS

All experiments were conducted in the Vancouver region of British Columbia, Canada. Experimental colonies were established from package bees imported from Vancouver Island, B.C., then a mite free area. Each package consisted of 1kg of bees and a queen of Australian origin, collected on 7 May and installed on 8 May. At installation, each colony received two frames of honey, sugar syrup *ad libidum*, and the same amount and type (worker, drone or foundation) of comb. Colony treatments were assigned to 40 colonies, but due to inclement weather, colonies were inaccessible until after several had swarmed. After colonies that swarmed were removed, treatments were: no mites (n = 8), varroa mites (n = 4), tracheal mites (n = 9) and both mites (n = 6). Tracheal mite infestations were established by taking bees from colonies with known tracheal mite infestations (about 50%), pooling them in a large screen box and adding 400g of these bees to each colony on 20 May. Colonies that were not in treatment groups with tracheal mites received 400g of bees from uninfested colonies. The introduced bees were sprayed lightly with dilute sugar syrup and placed on newspaper atop the frames of a colony. Varroa infestations were established by removing frames of bees near eclosion from colonies known to have high varroa levels, placing them in an incubator and collecting newly emerged bees with varroa mites into petri dishes.

These petri dishes were opened and inverted on the top bars of the colony, so the bees could crawl in and introduce mites. In this fashion, colonies in treatment groups that required varroa infestation received 86 mites each on 20 May.

Both mites spread rapidly within an apiary, so to preserve treatment integrity Apistan strips were placed in colonies that were not to have varroa mites and menthol and grease patties were applied to colonies that were to be tracheal-mite free. Menthol was applied once on 11 June, and the other treatments were applied in the spring and fall 1997 and 1998. Apistan does not impact transfer or reproduction of tracheal mite populations (Pettis et. al. 1988), and although menthol vapor may impact varroa mites (Imdorf et al. 1995) it is unknown if the menthol or grease patties as applied had an impact on varroa. Because varroa populations were successfully established, menthol and grease effects were considered negligible. Colonies also were fed sugar syrup medicated with antibiotics (terramycin and fumagillin) to prevent foulbrood and nosema, and pollen supplements were given at installation and again in February 1998. Apistan, menthol and grease patties have no effects on colony productivity or bee behavior (Westcott and Winston, 1999).

Each colony was assessed three times, 18 August 1997, 25 March 1998 and 24 July 1998. Measurements were taken by holding a plastic transparent grid divided into 32 equal squares over a frame and tabulating the number of grid squares of sealed worker brood area, sealed drone area, honey, pollen, and adult

bees. Adult bees were measured before daily foraging started. Colonies also were weighed at assessment, and mite populations sampled. Tracheal mite sampling was done by collecting bees from each colony into ethanol, dissecting 30 of them under a microscope, and inspecting the prothoracic trachea for mites. Varroa sampling was done with sticky boards, placing a piece of corrugated plastic coated with a petroleum jelly/vegetable oil (1:1 vol) blend covered with a plastic mesh screen in the bottom of each hive. This board was removed from the hive after 24 hours to count the mites that fell and were trapped. Colonies were sampled in between Apistan treatments, "mite drop" reflects natural levels in all measurements. Colony mortality was assessed every 2-3 weeks by opening the colonies.

Statistical analysis was done in SAS 6.12 and SAS JMP IN 3.1.5. Mite treatments were compared among two groups, colonies seeded with a mite species and those not seeded. One-way ANOVAs were then done for varroa and tracheal mites on each date. Colony productivity was analyzed using a one-way ANOVA for each parameter, dates were analyzed separately. Survivorship curves were compared using a Lifetest procedure in SAS.

RESULTS

Mite treatments were successfully established using the seeding method, but by the end of the experiment there was considerable tracheal mite drift.

Tracheal mite populations in seeded colonies were significantly higher than in un-seeded colonies for all assessment dates except the last one (Figure 1). Varroa mite populations were always significantly higher in colonies seeded with varroa than in un-seeded colonies (Figure 2).

There was no colony mortality during the first season, but on the March assessment (after the first winter), 83 % of colonies with both mites were dead (Figure 3A). On the following assessment, there was 50 % mortality in the varroa colonies, and one queen loss in the colonies with no mites. In September 1998, another varroa colony died, bringing varroa treatment mortality to 75% (Figure 3B). Probability of survival was significantly different among treatment groups (Lifetest, $p < 0.0001$, Chi-Sq = 23.72), colonies with both mites died first, followed by colonies with varroa, and the only other mortality (one colony in the no mite treatment) was due to queen loss.

In the first year, colonies with both mites had significantly more honey than colonies with no mites by August, and single mite treatments had intermediate honey stores (Figure 5, $p = 0.0330$, $F = 3.4553$). No other parameters were significantly affected by mite treatment until after winter (Figure 4, Figure 5). In March, most colonies with both mites were dead, so only the three remaining treatments were compared. Colonies with varroa had significantly less worker brood ($p = 0.0003$, $F = 13.7140$) (Figure 4), and fewer adult bees ($p = 0.0261$, $F = 4.5503$) (Figure 4). Colonies with varroa had more

honey than colonies with no mites, and tracheal mite colonies had intermediate honey stores ($p = 0.0194$, $F = 5.0142$) (Figure 5). On the final assessment, in July, only two treatments were compared because two of the four varroa colonies were dead. There were no significant differences in any of the parameters measured between uninfested and tracheal mite colonies, but at this point there were statistically indistinguishable tracheal mite populations established in both treatments (Figure 1).

DISCUSSION

Colonies infested with both mites sustained considerably greater mortality over the winter than colonies with just one mite or uninfested colonies. Most colonies with varroa died over the next few months, while tracheal mite and no mite colonies survived. These results support the hypothesis of a synergistic effect of tracheal and varroa mites on colony mortality. Tracheal mites often are ignored by beekeepers, but our results indicate that beekeepers should be diligent about detecting and controlling both mites.

Mortality due to mites often has been discussed in relation to climate, and it is interesting that mortality was high in the mild climate of Vancouver, B.C., with rainy winters and little snow. Average coastal temperatures are much warmer than most of Canada and the U.S. at similar longitudes, and if mites were causing death under these conditions, mortality would have likely been much higher under

more severe winter conditions. Thus, some of the impact attributed to varroa in other regions could be due to dual mite infestations. If so, controlling tracheal mites could reduce varroa impact. As varroa becomes resistant to fluvalinate (Elzen, et al. 1998) it will be increasingly important to reduce the impact of varroa infestation by using integrated approaches.

Bee stock should be selected in terms of dual infestation rather than one mite at a time, because of the combined effect. Bees have been selected and bred to tolerate tracheal mite infestation with some success (reviewed in Wilson et al. 1997) but stock that is tracheal mite tolerant, supporting a sub-lethal tracheal mite infestation, could be more vulnerable to dual infestations than stock requiring tracheal mite treatment. Honey bee pathology requires a more holistic, integrated approach, considering the many risks and stresses on any one colony; varroa mites and tracheal mites are just two of many possible threats. Research on the impact of varroa or tracheal mites should include assessment of the other mite to avoid misinterpreting results confounded by dual infestation.

Comparisons of colony productivity show that in the first season, colonies with both mites had more honey than colonies with no mites. Perhaps mite stress causes colonies to gather more resources. However, no similar effect was seen in a study comparing individual forager behavior among bees with tracheal and varroa mite parasitism (Downey, in press.). Alternatively, colonies with mites may use less honey, due to lower population size, but this difference was not

reflected in brood or adult population measurements.

Varroa infestation clearly was associated with less brood, fewer bees and more honey by spring, one year after establishment. These colonies were failing, and smaller adult and brood populations resulted in less honey consumption and higher stores. Pollen stores were lower, although statistically this effect was not quite significant ($p = 0.06$, $F = 3.3603$). This result is consistent with recent studies by Janmaat (in prep.) indicating that colonies with varroa mites are less efficient pollen users, using more pollen to produce equal or lower amounts of brood. Tracheal mites colonies also had more honey at this point, possibly a result of using less honey through the winter. Studies by Nasr et al. (1999) indicate that bees with tracheal mites are not able to respire as effectively as uninfested bees to maintian high core temperatures in winter, which would result in less honey consumption.

Tracheal mites often are overlooked as an inconsequential threat, and in our mild climate colonies with tracheal mites performed comparably to uninfested colonies. However, tracheal mites in colder climates may be more problematic and our study revealed that tracheal and varroa mites together in a colony present a much greater mortality risk than either mite alone. As the parasite and pathogen pressure on managed honey bee colonies continues to mount, innovative controls are needed to develop integrated pest management (IPM) systems for honey bee colonies. This study demonstrates that dual mite infestation suggests a bleak

future for a honey bee colony. Colony management and future mite research should consider not just varroa, but both mite species and possibly interactions with other bee diseases.

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DETAILED ABSTRACT

Colony mortality and productivity were compared between honey bee (*Apis mellifera* L) colonies infested by zero, one or both species of parasitic mites (*Acarapis woodi,* the honey bee tracheal mite, or *Varroa jacobsoni*, varroa). Colonies were started from 1 kg. packages of bees, bees carrying varroa or tracheal mites were added to establish treatment groups, colonies were treated to maintain only the appropriate mite species, and then colony mortality was monitored for 16 months. Periodic assessments also were done to measure adult bee population, sealed worker brood, sealed drone brood, colony weight, honey stores, pollen stores and mite levels. Mite populations were established and maintained although there was some contamination (Figures 1 & 2). There was no colony mortality until March after the first winter, when 83% of colonies with both mites were dead. By the termination of the experiment in September, 75% of varroa colonies were dead, one colony with neither mite was dead due to queen loss, and no tracheal mite colonies died (Figure 3). In the first season, colonies with tracheal and varroa mites initially had more honey stores (Figure 5), but by spring, when colonies with both mites were dead, the varroa colonies had significantly less worker brood, fewer adult bees (Figure 4) and more honey than colonies with no mites or tracheal mites. In the last assessment, when varroa colonies were removed due to mortality, colonies with tracheal mites and no mites were statistically indistinguishable on all productivity parameters measured, but at

this time tracheal mite populations were established in all colonies. These results indicate a synergistic interaction between tracheal and varroa mites with regard to colony mortality, and even if tracheal mites alone are not having an impact, treatments against tracheal mites should be applied if there is a risk of dual infestation.

FIGURE LEGENDS

Figure 1: Tracheal mite infestation levels (mean ± SE) in colonies seeded with tracheal mites and those not seeded. Letters indicate means that were significantly different ($p < 0.05$) using Tukey-Kramer's test.

Figure 2: Varroa mite drop measured for 24 hours with no acaricide (mean ± SE) in colonies seeded with varroa and those not seeded. Letters indicate means that were significantly different ($p < 0.05$) using Tukey-Kramer's test.

Figure 3: Colony mortality (%) in March and September 1998 (Lifetest, $p < 0.001$).

Figure 4: Adult bee population, sealed worker and drone brood analyzed separately with one-way ANOVAs on each date. Letters indicate means that are significantly different ($p < 0.05$) using Tukey-Kramer's test.

Figure 5: Colony weight, honey and pollen stores analyzed separately with one-way ANOVAs on each date. Letters indicate means that are significantly different ($p < 0.05$) using Tukey-Kramer's test.

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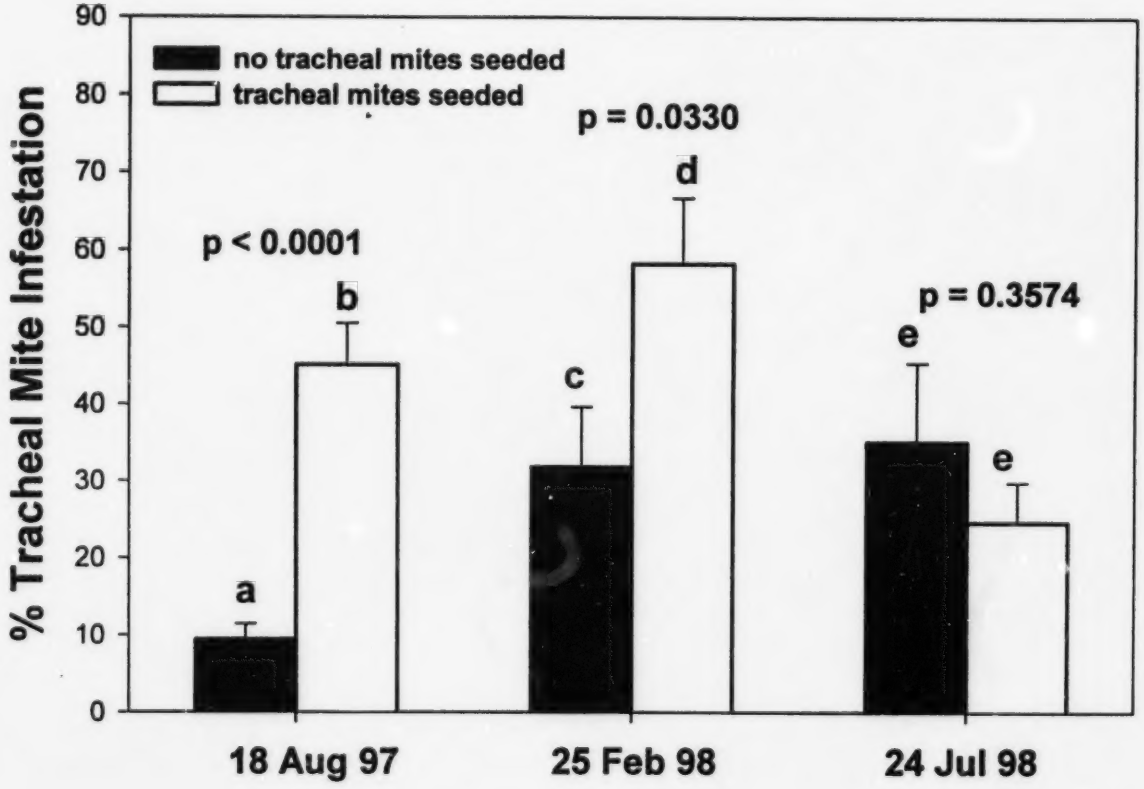
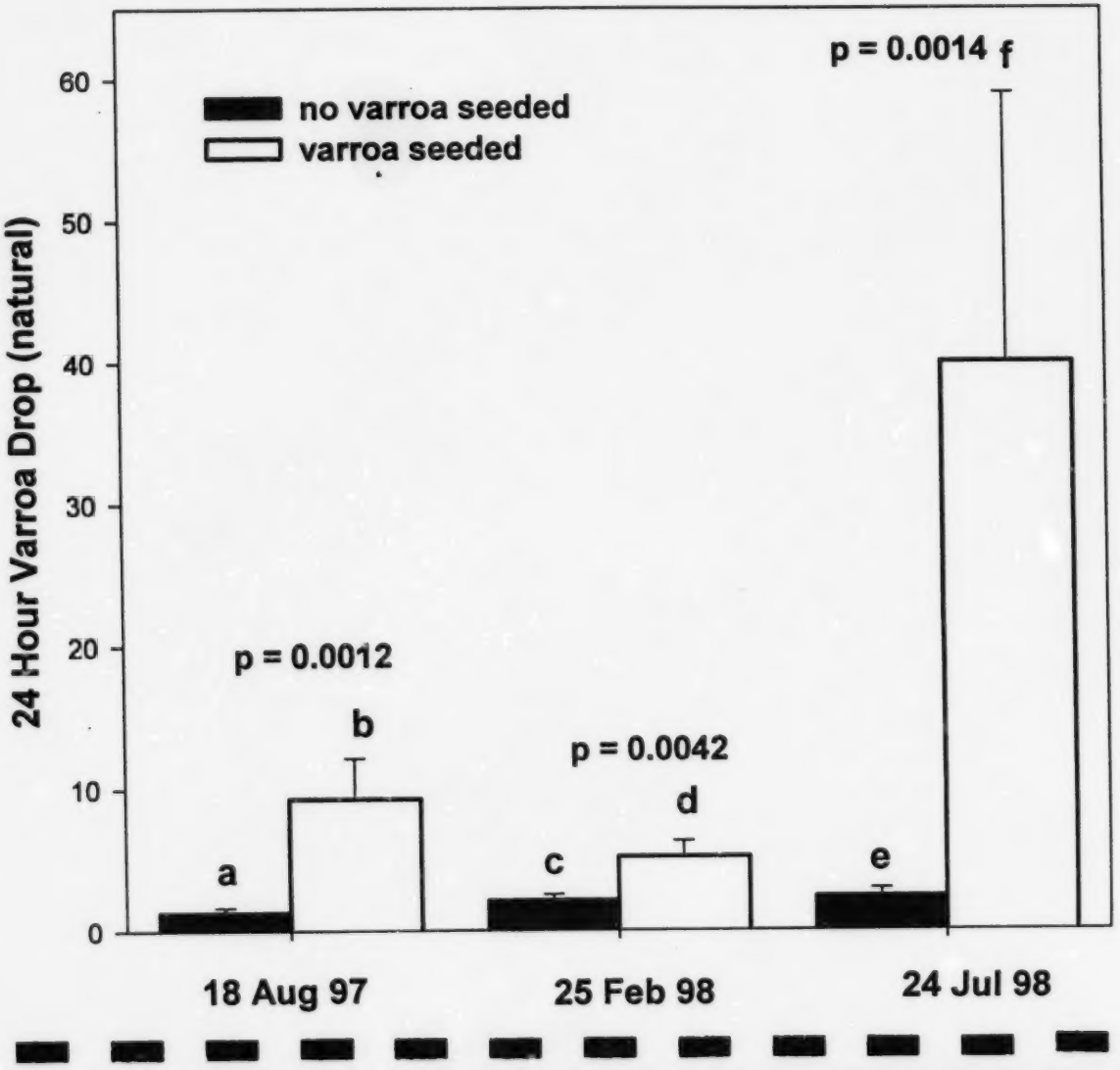
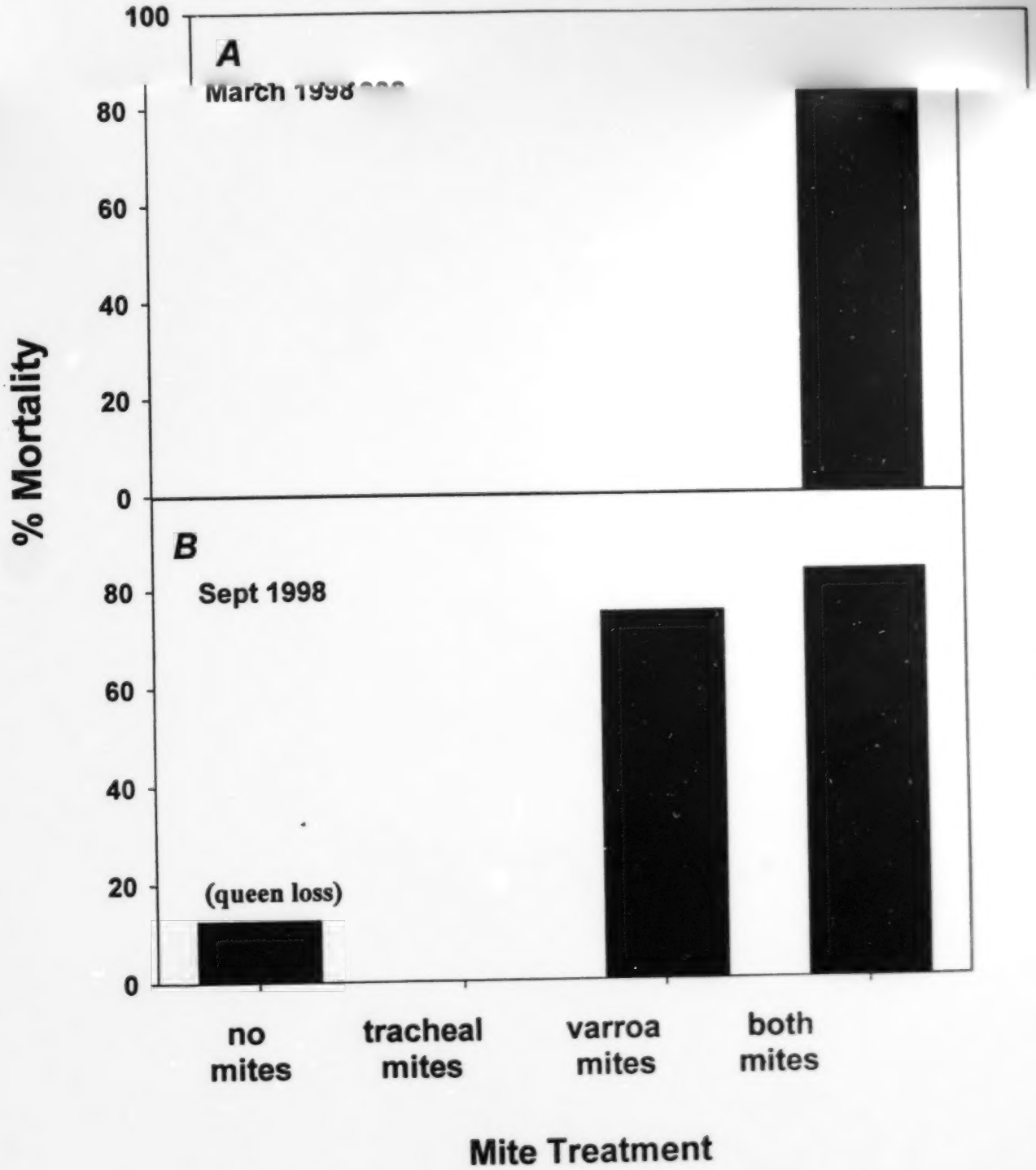
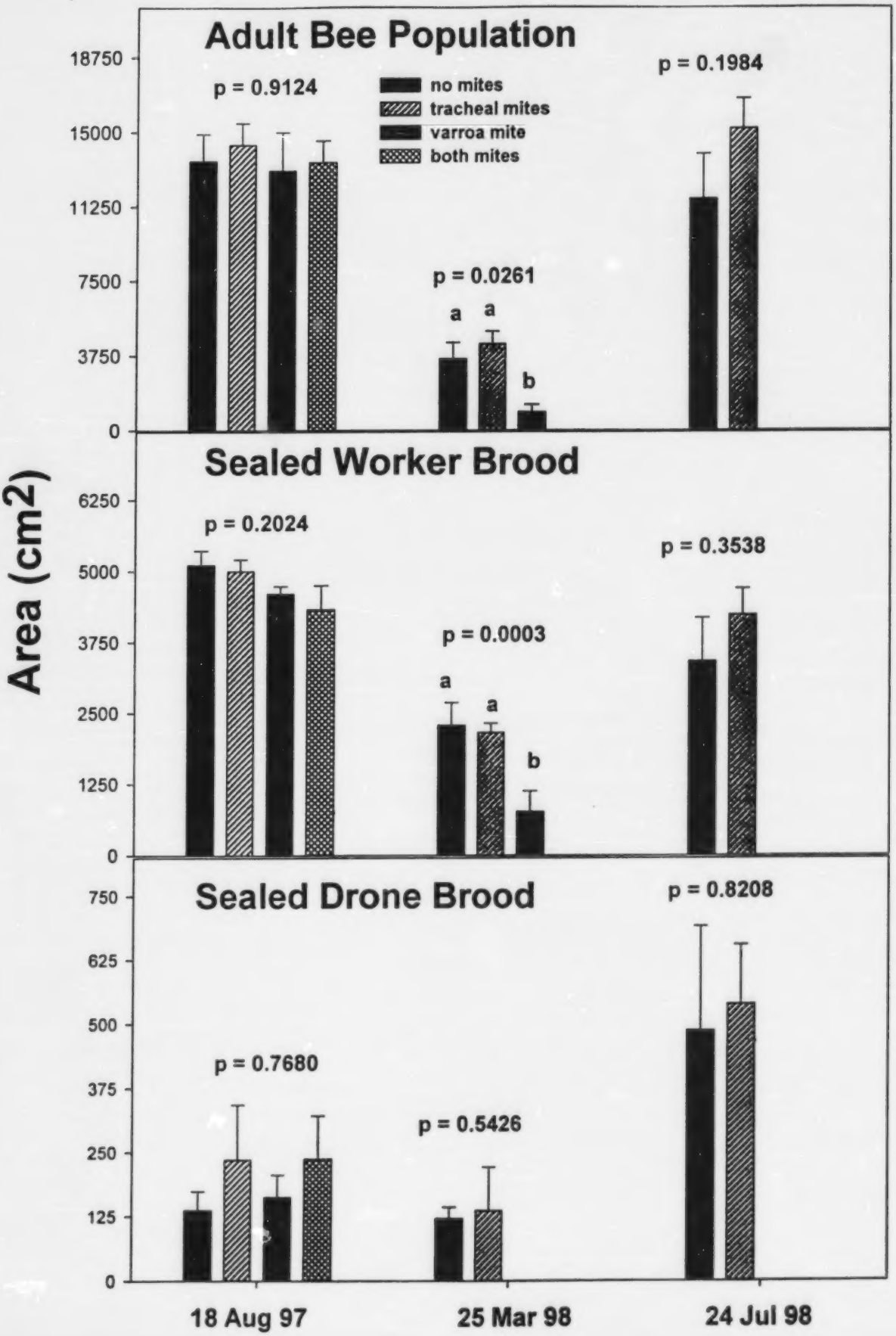
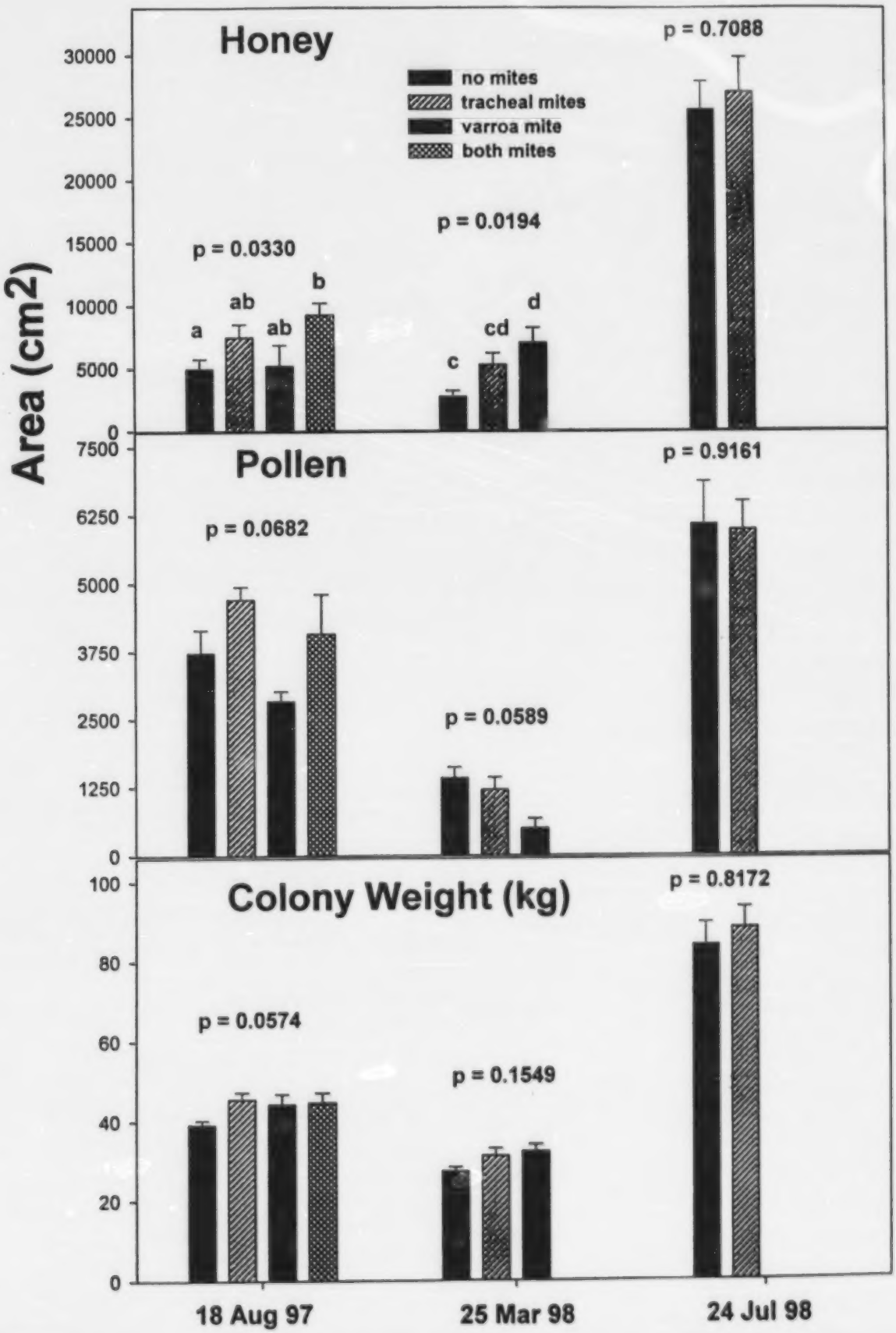

% Tracheal Mite Infestation
90
80
70
60
50
40
30
20
10
0
no tracheal mites seeded
tracheal mites seeded
p < 0.0001
p = 0.0330
p = 0.3574
a
b
c
d
e
e
18 Aug 97
25 Feb 98
24 Jul 98


p = 0.0014
f
no varroa seeded
varroa seeded
60
50
40
30
20
10
0
24 Hour Varroa Drop (natural)
p = 0.0012
b
p = 0.0042
d
a
c
e
18 Aug 97
25 Feb 98
24 Jul 98


100
A
March 1998
80
60
40
20
0
% Mortality
B
Sept 1998
80
60
40
20
0
(queen loss)
no mites
tracheal mites
varroa mites
both mites
Mite Treatment


Adult Bee Population
p = 0.9124
p = 0.0261
p = 0.1984
no mites
tracheal mites
varroa mite
both mites
a
a
b
18750
15000
11250
7500
3750
0
Sealed Worker Brood
p = 0.2024
p = 0.0003
p = 0.3538
a
a
b
6250
5000
3750
2500
1250
0
Sealed Drone Brood
p = 0.7680
p = 0.5426
p = 0.8208
750
625
500
375
250
125
0
Area (cm2)
18 Aug 97
25 Mar 98
24 Jul 98


Honey
Pollen
Colony Weight (kg)
Area (cm2)
no mites
tracheal mites
varroa mite
both mites
p = 0.0330
p = 0.0194
p = 0.7088
p = 0.0682
p = 0.0589
p = 0.9161
p = 0.0574
p = 0.1549
p = 0.8172
a
ab
ab
b
c
cd
d
30000
25000
20000
15000
10000
5000
0
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Single and Dual Parasitic Mite Infestations on the Honey Bee, *Apis mellifera* L.

Running Head: Dual mite parasitism of honey bees

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ABSTRACT

The onset of foraging, proportion of pollen collectors, and weight of pollen loads were compared in individual honey bees (*Apis mellifera*) infested by zero, one (*Acarapis woodi*, the honey bee tracheal mite, or *Varroa jacobsoni*, varroa), or both species of parasitic mites. Phoretic varroa host choice also was compared between bees with and without tracheal mites, and tracheal mite infestation of hosts was compared between bees parasitized or not by varroa during development. The proportion of pollen collectors was not significantly different between treatments, but bees parasitized by both mites had significantly smaller pollen loads than uninfested bees. Mean onset of foraging was earliest for bees parasitized by varroa during development, 15.9 days. Bees with tracheal mites began foraging latest, at 20.5 days, and foraging ages were intermediate in bees with no mites and both, 17.6 and 18.0 days respectively. Phoretic varroa were found equally on bees with and without tracheal mite infestations, but bees parasitized by varroa during development were almost twice as likely to have tracheal mite infestations as bees with no varroa parasitism, 63.9% and 35.5%, respectively. These results indicate that these two parasites can have a biological interaction at the level of individual bees that is detrimental to their host colonies.

INTRODUCTION

Honey bee (*Apis mellifera* L.) colonies are a rich and stable habitat for parasites. Host bees, honey, pollen and wax are present year-round, and bees regulate temperature, humidity and carbon dioxide levels within narrow limits (Winston, 1987). As a consequence, honey bee colonies are exploited by an array of parasites, pathogens and associated organisms (Bailey and Ball, 1991), which may be an important selective force on the evolution of colony structure and function (Gadagkar, 1992; Sherman et al. 1988; Shykoff and Schmid-Hempel, 1991a,b). Over 40 mite species are known to be associated with honey bee colonies (Eickwort, 1988), several of which are parasitic, including two that cause severe bee colony mortality and world-wide losses in the beekeeping industry (Bailey and Ball, 1991). The impact of these two mites, *Acarapis woodi* (Rennie) and *Varroa jacobsoni* Oudemans, on individual honey bees is the focus of this study.

Varroa and tracheal mites feed on honey bee hemolymph, depriving the bee of hemolymph and protein (Bailey and Ball 1991; De Jong et al., 1982; Kovac and Crailsheim, 1988). This effect is usually sub-lethal to the individual bee, but infestation by either mite species can kill a bee colony when not controlled. Although the direct mechanism of death is not known, both mites have been associated with higher levels of bacterial and viral pathogens in bee hemolymph, and it is suspected that mite infestation decreases adult longevity due to these pathogens (Allen and Ball 1996). Overall, varroa mites are more virulent than tracheal mites, killing a colony within 1-2 years if left unchecked (Bailey and Ball, 1991). Tracheal mite infestations may last for many years without noticeable effects.

The presence of both mites in honey bee colonies is a critical management problem facing beekeepers world-wide, but also provides a fascinating opportunity to explore host-

parasite interactions in a social insect. Of particular interest are questions involving how single or dual-species mite infestations might affect individual performance in areas that require integration in colony-level functions, such as foraging. For honey bees, the labor expended to gather nectar and pollen varies at both individual bee and colony levels, due to seasonal, nutritional, heritable, and colony-state factors (Schmid-Hempel et al., 1993). Several stress events, such as wax deprivation (Fergusson and Winston, 1988), depletion of nectar and pollen stores (Rinderer, 1982; Fewell and Winston, 1992) and worker loss (Winston and Fergusson, 1985) induce resource-gathering responses in honey bee colonies, including increased number of foragers, accelerated task ontogeny (i.e. earlier onset of foraging), or greater effort by individual foragers (foragers carry larger loads or visit more flowers) (Schmid-Hempel et al. 1993). In addition to resource deprivation, stresses that cause precocious foraging include infection with diseases, such as *Nosema apis* (Wang and Moeller, 1970), Sacbrood virus (Bailey and Fernando, 1972), and hemolymph removal (Huang, personal communication). Infestation with either varroa or tracheal mites might induce similar responses, and dual parasitism could have additive or even synergistic effects on foraging behavior.

Dual mite infestations also provide an opportunity to determine whether the presence of one mite influences host choice or survival by the other. Phoretic varroa, those between reproductive bouts on pupae, and host seeking tracheal mites both prefer young adult *A. mellifera* hosts. Tracheal mites are more dependent on their initial host bee, as they spend their life cycle within tracheal tubes and only their offspring exit to choose a new host, in contrast to the highly mobile varroa which easily switch adult hosts. Tracheal mites use surface chemicals to identify young bee hosts (Phelan et al., 1991; Lee, 1963; Gary et al., 1989). Varroa mites probably use aliphatic alcohols and aldehydes found in honey bee cocoons to identify appropriate hosts for

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reproduction (Donze et al., 1998). Both mite species are known to *A. cerana* and *A. mellifera*, and parasitism may affect a bee's attractiveness as a new host, but it is not known whether parasitism by one species of mite affects host selection by the other species. Infestation by two species of parasitic mites allows examination of the interface of two parasites in a complex multiorganismal system, in addition to its implications for honey bee management. The effect of single species infestations have been studied for several years, but no studies have been conducted to examine the impact of single vs. dual infestations on individual honey bees and honey bee colonies, or the impact of one mite on the other. The main objectives of this project were 1) to compare the impact of tracheal and varroa mites, independently and together, on foraging parameters of individual bees, and 2) to investigate the sensitivity of each mite species to the presence of the other during host selection.

METHODS AND MATERIALS

Onset of Foraging, Pollen Collection and Pollen Load Weight

Experiments were conducted over two summers, 1996 and 1997, in Burnaby, B.C. Canada. Bees that had been fed on by varroa during the bee's pupal stage were obtained in two ways. In June 1996 (Round I) and July 1997 (Round II), a single female varroa mite was inserted into a newly sealed cell containing a larva. Cell cappings were cut open using a disposable scalpel, and varroa were lifted onto a fine paintbrush and allowed to crawl into the cell. The cell capping was tamped back down after insertion. Time of capping was monitored by mapping brood cells 12 h before varroa were to be inserted. Control bees were obtained by performing the same procedure without inserting a varroa mite. Varroa were collected by shaking young bees from a heavily infested colony in icing sugar (Boecking and Ritter, 1993), which causes the mites to drop off host bees. Varroa came from colonies maintained to have very high

varroa populations, about 1 km away from the healthy colonies with low varroa infestation from which brood were taken. Cells were seeded in this manner on eight frames, each mapped using a plastic transparency tacked to the frame (Ifantidis, 1983). In August 1997 (Round III) brood frames containing larvae near capping stage were moved from a healthy colony to one heavily infested with varroa, and many cells were naturally infested. Control bees emerged from uninfested cells on the same frames.

Frames from both techniques were placed in a healthy colony with low mite levels to complete bee development. One to two days before emergence, the frames were placed into a hot room (32° C) and emerging bees were captured and marked with colored and numbered bee tags glued to their thoraces, noting the number of mites that had fed on them during development. Each bee was placed immediately onto a frame of bees taken from the colony it would live its experimental life in, and the frames replaced into the colony every 24 hours. This frame rotation ensured immediate bee exposure to tracheal mites.

Each tracheal mite-infested experimental colony had pre-monitored tracheal mite prevalence between 20-60%, determined by dissecting 30 bees collected from the inner cover (or frames nearby) in 70% ethanol and inspecting for tracheal mite presence or absence. All colonies were treated with fluvalinate (Apistan) prior to the experiment, which kills >99% of varroa mites but does not affect tracheal mites. In these colonies, tagged bees (either with or without varroa parasitism during development) were exposed to tracheal mites. Thus each marked bee was in one of four treatment groups: bees with 1) no mites, 2) varroa only, 3) tracheal mites only, or 4) varroa and tracheal mites.

To measure the onset of foraging, experimental bees were divided among six colonies prepared 1-2 weeks before the experiment began. In 1996 (Round I), three colonies were split

from each of two large parent colonies. In 1997 (Rounds II and III), the experimental colonies had six different parent colonies. The parent colonies had 10-50% tracheal mite prevalence, which was monitored prior to and after the experiment (sampling method described above). Each five frame colony had 2-3 frames of bees, one frame of brood, one frame with pollen and two frames with honey. The colonies were given open mated sister queens (closest to *A. m. ligustica* in origin). After tagged bees were introduced, colony entrances were closed with a screen twice every day, once between 10:30-12:00, and again between 13:30-16:00, for either 15 minutes (Rounds I and II) or 30 minutes (Round III) each time. Any returning tagged bees were assumed to be first time foragers, and were collected and dissected to examine for tracheal mites. Foragers were classified as pollen foragers if they had pollen loads visible to the naked eye, otherwise they were classified as non-pollen foragers. In Rounds II and III, pollen loads were removed with forceps, and weighed (wet weight). Because there is an important genetic component of pollen collection (Page et al., 1995) and foraging onset (Giray and Robinson, 1994), bees from various genetic sources in Round I were assigned randomly to a colony by pooling bees and distributing them to each of the six colonies. In Rounds II and III, bees were mixed and distributed the same way, and the colony source of the bees was recorded to better assess the genetic effect.

Mite Host Choice

To compare tracheal mite infestation of hosts with and without varroa during development, varroa-parasitized and unparasitized bees were obtained using the second method described above, placing larvae near capping stage in a colony with high varroa levels. Six colonies with one frame of eggs and young larvae, one frame of honey, and a queen were established two weeks prior to the introduction of these bees. Each colony received three cups of

a homogenous mixture of bees shaken from colonies with various tracheal mite infestation levels expected to average 40%. Paint-marked bees with and without varroa parasitism during development were introduced; adult varroa were not removed. Seven days later, these bees were collected and dissected to inspect for tracheal mites.

Phoretic varroa host preference was compared among same-age bees with and without tracheal mites. Frames of emerging bees were taken from colonies with low mite levels and placed in a 34° C incubator overnight. From these frames, 1200 newly emerged bees were paint-marked and divided randomly and evenly among the six colonies above. These colonies had tracheal mite prevalence between 40-56%. Varroa then were introduced to these colonies. Frames of emerging bees were taken from colonies heavily infested with varroa, placed in a 34° C hot room, and newly emerged bees were inspected for varroa. Each colony received approximately 400 phoretic varroa, no brood were proper age for the varroa to enter and reproduce. When the marked cohort reached eight days of age, the bees were collected in two groups, with and without phoretic varroa, and dissected to compare tracheal mite infestation levels.

Statistical analysis for onset of foraging, pollen collection, pollen load weight and mite host choice were performed in SAS JMP IN 3.1.5. The onset of foraging was analyzed using a one way ANOVA and Duncan's Multiple Comparison Test. The genetic effect on the onset of foraging was analyzed using a two-way ANOVA. Pollen collection among mite treatments and mite host choice were compared using Chi-Square Tests, and pollen load weights were compared among mite treatments using a General Linear Model with Orthogonal Contrasts.

RESULTS

Onset of Foraging, Pollen Collection and Pollen Load Weight

The mean age when workers began to forage was significantly different between mite treatments ($F = 12.17$, d.f = 3, $p < 0.005$, $N = 1006$). Bees infested with varroa as pupae foraged significantly earlier (15.9 days of age) than bees with tracheal mites (20.5 days). The foraging age of bees with no mites (18.0 days) was less than bees with tracheal mites but not significantly different from bees with both species (Figure 1). Foraging age decreased with the severity of parasitism by varroa mites during development but the relationship was very weak (Figure 2). There also was a genetic effect on the onset of foraging , ($F = 5.20$, d.f. = 11, $p = 0.0001$), with means ranging from 13.5-24.0 days with genetic source (Rounds II and III). Foraging began at significantly different ages in Rounds I-III (21.2, 18.6, and 15.1 days, respectively) ($F = 47.06$, d.f. = 2, $p < 0.0001$).

The proportion of bees collecting pollen was not significantly different among treatment groups: no mites 36.8%, tracheal mite 36.3%, varroa mite 32.3%, and both mites 30.7% ($X^2 = 2.793$, $p = 0.425$, d.f. = 3, $N = 1006$). Pollen collection did vary with the genetic source of the bees: range = 21.2 – 43.8% pollen foragers ($X^2 = 17.46$, $p = 0.0146$, d.f. = 7, $N = 8$). The percentage of returning marked bees with pollen loads was highest in June 1996 (38%, $N = 439$) lower in July 1997 (36%, $N = 335$) and was lowest in August 1997 (28%, $N = 247$) ($X^2 = 7.88$, $p = 0.0195$, d.f. = 2, $N = 3$).

Pollen load weight was significantly lower in dually infested bees than in uninfested bees, (Figure 3) ($F = 6.077$, d.f. = 1, $p = 0.0147$,). Genetic source had a significant impact on pollen load weight ($F = 2.15$, d.f. = 8, $p = 0.041$), with the range of pollen load weights (mean ± SE) 4.6 ± 1.0 to 8.6 ± 0.9 mg.

Mite Host Choice

Tracheal mites infested bees with and without phoretic varroa at statistically

indistinguishable levels (33.3%, N = 144 and 28.9%, N = 204, respectively) (Figure 4A). Tracheal mites were found almost twice as often in bees that had been parasitized by varroa during development (N = 108, 63.9% with tracheal mites) than in bees without varroa during development (N = 197, 35.5% with tracheal mites) (Figure 4B). In the foraging trials, bees with varroa parasitism were 76.9% infested with tracheal mites, significantly higher than in bees without varroa (64.2%) (X^2= 15.361, $p < 0.001$, d.f. = 1, N = 1006), despite the fact that both groups of bees in Round I were 90% infested with tracheal mites. Excluding data for Round I gave 64.8% tracheal mite infestation in bees parasitized by varroa and 46.8% tracheal mite infestation in bees with no varroa parasitism (X^2= 14.029, $p < 0.001$, d.f. = 1, N = 570). Tracheal mite infestation did not vary significantly with genetic source of the bees (X^2= 10.88, p = 0.1438, d.f. = 7, n = 556).

DISCUSSION

The most surprising result of this study was that the two mite species alone had *opposite* effects on the ages at which bees began foraging, while bees infested with both mites showed intermediate foraging ages, equal to unparasitized bees. The decreased foraging age of bees infested by varroa as pupae was the more predictable result, since a generalized honey bee response to stress is to increase foraging effort and accelerate temporal caste ontogeny, (Schmid-Hempel et al., 1993). These stress responses maximize immediate resource collection, but bees that forage sooner will die younger due to mortality factors such as predation, inclement weather, and physiological aging, so that the cost of accelerated ontogeny is higher than that of increased effort by individuals already foraging (Fergusson and Winston, 1988).

Varroa induces precocious foraging (Schneider, 1986) (Figure 1), and this effect is amplified by mite reproduction and multiple infestation, since foraging begins even earlier

(Figure 2). Fergusson and Winston (1988) document a graded honey bee stress response, whereby lesser stresses cause foragers to increase their efforts, and greater stress causes precocious foraging. In the present study, varroa parasitism did cause accelerated ontogeny but bees parasitized by varroa were not more likely to collect pollen, nor did they have bigger pollen loads.

The differences we observed in foraging ages suggest that parasitism by varroa could be used to explore the interaction between physiological effects of stress on individuals and colony-level responses, especially involving temporal caste structure. Bees fed on by varroa during development emerge underweight (De Jong et al., 1982), and hemolymph volume and protein content are both substantially (15-50%) depleted (De Jong, 1990). Although adult bees can supplement protein levels by pollen feeding, protein deprivation during larval development could affect gland development, making these bees less suitable for in-hive tasks such as nursing. The effect we observed of earlier onset of foraging by bees that had been fed upon by varroa mites is consistent with the hypothesis that feeding by varroa during pupal development causes a more rapid increase in juvenile hormone (JH) titres in the adult bee. The relationship between JH titres and foraging has been well documented (Robinson 1992). The effect of varroa on JH is presently unknown and should be investigated.

In contrast, the delayed onset of foraging in bees parasitized by tracheal mites is unusual because no stress on honey bee colonies is known to retard task ontogeny. Gary and Page (1989) found that tracheal mite infestation caused no significant differences in number of foraging trips, frequency of foraging trips, round trip times, frequency of pollen collection, time between foraging trips, nectar loads and summer survival, although this study may not have captured the impact of tracheal mites due to low infestation levels. Foraging puts a bee at greater risk than in-

hive tasks, and delayed foraging might provide a safer environment for the many tracheal mites living in each bee. Delayed foraging could reflect host manipulation, and there are many other examples of host manipulation in social insects (Schmid-Hempel 1995). Tracheal mites also could alter individual fitness; impaired respiration or flight ability could make infested bees less suitable for foraging. The increased foraging age observed in tracheal mite-infested bees is also compatible with a possible effect of mite infestation on JH synthesis. Offsetting effects of infestation by the two mite species on JH production could explain the intermediate age at foraging onset for bees infested with both mites.

Their opposite impact on the onset of foraging may reflect differences in tracheal and varroa mite life history with regard to transmission. Varroa parasitism increases forager drift (Sakofski, 1990), mites can move quickly to a new host even if a drifting bee is not accepted in a foreign hive. In contrast, long-lived bees host large populations of tracheal mites (Pettis and Wilson, 1996) and this whole population of tracheal mites is jeopardized when that bee leaves the hive. Retarded ontogeny maximizes safety and longevity of tracheal mite host bees, and transmission is primarily in-hive, at night, between calm bees (Pettis et al., 1992). The opposite impact of these mites on the onset of foraging could reflect host manipulation.

Colony state variables can have an important role in individual foraging (Schmid-Hempel et al., 1993), and parent colony was a significant factor in Round I, and also in the experimental colonies. There is a genetic component to precocious foraging (Winston and Katz, 1982; Giray and Robinson, 1994) and pollen collection (Page et al., 1995), consistent with our results indicating that the genetic source of the bee was a significant factor in the onset of foraging, propensity to collect pollen and pollen load weight.

Pollen loads were smaller in dually infested bees than in uninfested bees, but this was not

coupled with greater proportions of foragers due to infestation. Thus, dual infestation may hinder a colony's ability to collect pollen. Other studies suggest that varroa parasitism also reduces colony efficiency at using pollen to rear brood (Janmaat, 1998), an adverse impact that would not be observed as foraging behavior but could have a profound impact on colony productivity and success.

Host selection by phoretic varroa was not affected by tracheal mite presence, however tracheal mites were found almost twice as often in bees that had been fed on by varroa during development. This implies that varroa parasitism may alter a bee in a way that makes it a more frequent tracheal mite target, or in ways that would enhance tracheal mite survival once they locate a host bee. Varroa parasitism during the pupal stage might change the external chemical profile of an adult bee, alter the bee's internal chemistry, or change it's ability to autogroom, an activity associated with lower tracheal mite levels (Pettis and Pankiw, 1994, Danka and Villa, 1998). Tracheal mites disperse nocturnally, likely because bees are in colonies and less active at night (Pettis et al., 1992). If varroa parasitism causes bees to further decrease activity, they could be more vulnerable to tracheal mite infestation. More research is needed to understand tracheal mite host selection, acceptance, and successful infestation to elucidate the mechanism underlying this result.

Although effect varies, each of these mites can be devastating to a honey bee colony. This study focused on individual bees to observe the interactive aspects of these parasites sharing a host. Colony-level studies measuring productivity and mortality with regard to dual infestation also indicate a colony-level synergistic effect in addition to the individual effects, with significantly higher colony mortality with dual compared to single species infestations. (Downey, in prep).

Our results have important implications for understanding colony resiliency to parasitic mite stress and host choice by parasites. In general, parasite and pathogen pressure on social insects can affect the evolution of colony structure and maintenance of social organization. Examining the effects of parasitic mites on colony functions such as foraging can expose the underlying integrative response mechanisms of parasitised individuals *and* the resulting colony condition. As varroa and tracheal mites continue to share host bees and colonies, honey bees can be used to answer many interesting questions regarding parasite interactions and host sharing. The complexity of social insect behavior and the lack of information regarding these parasites leaves many opportunities for further study.

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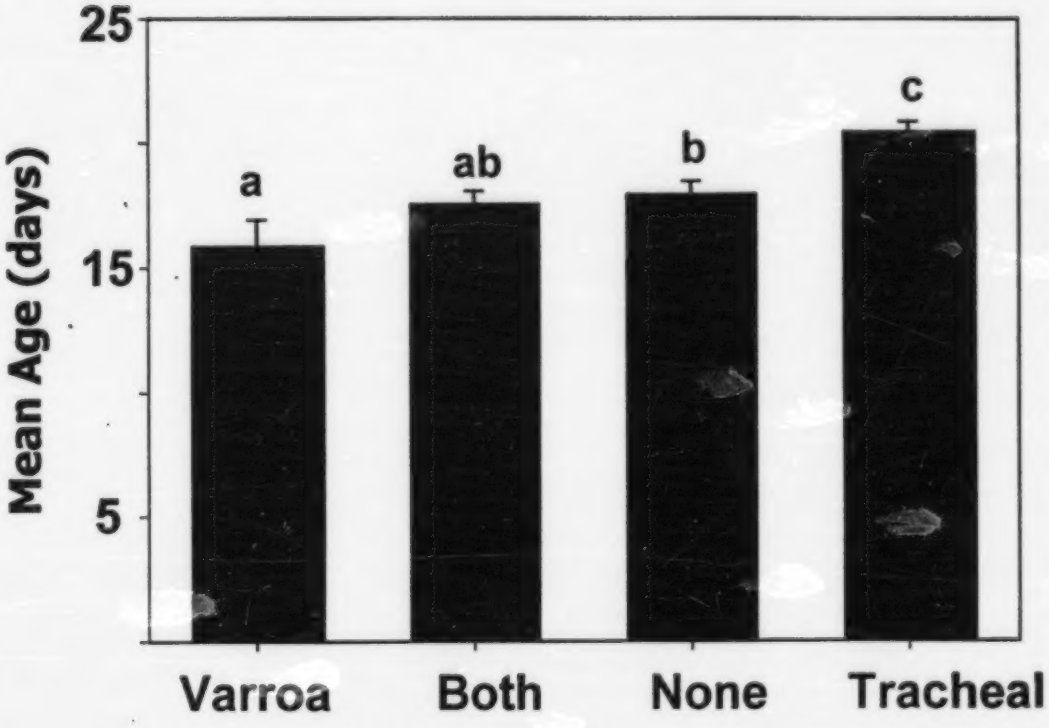
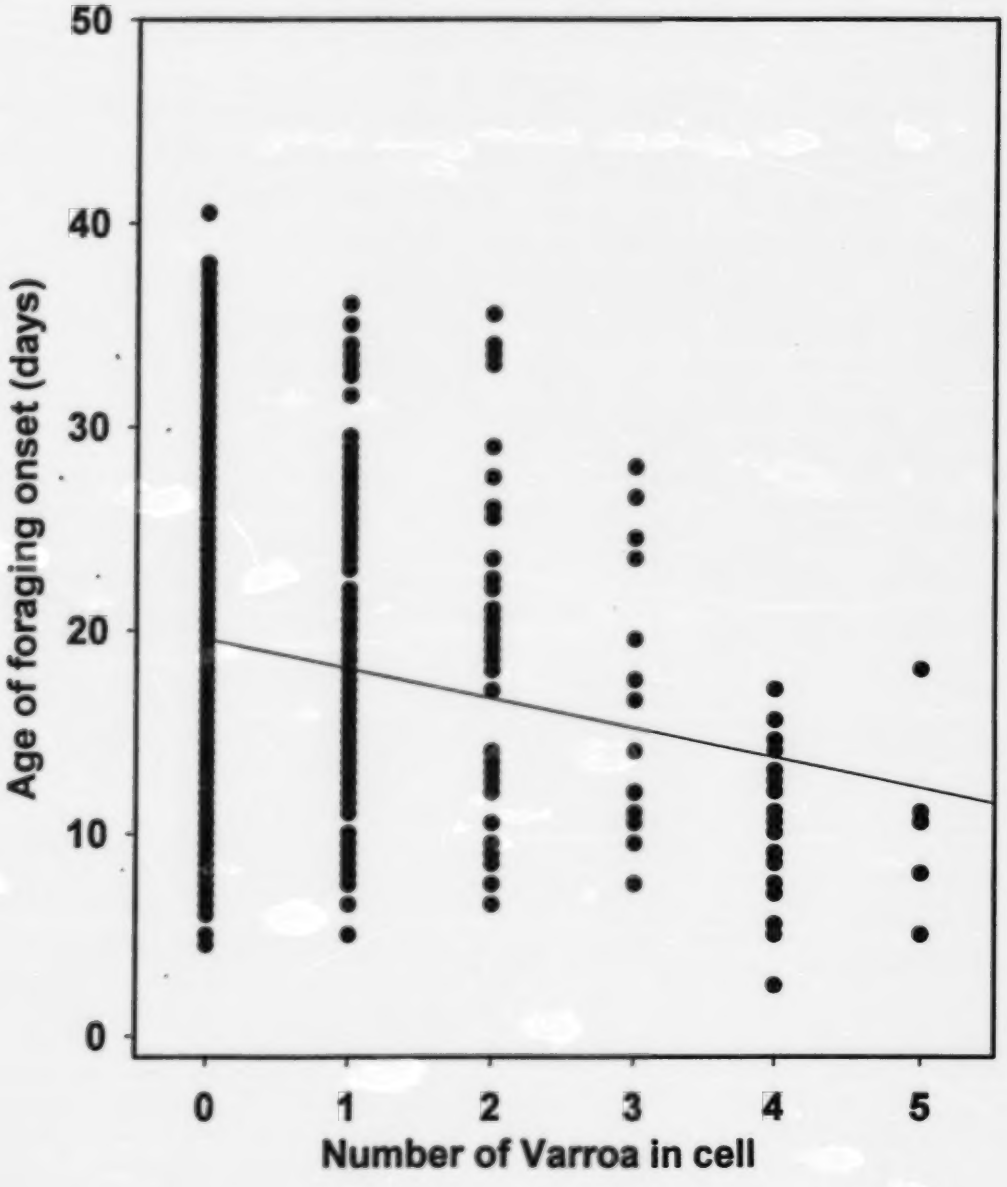
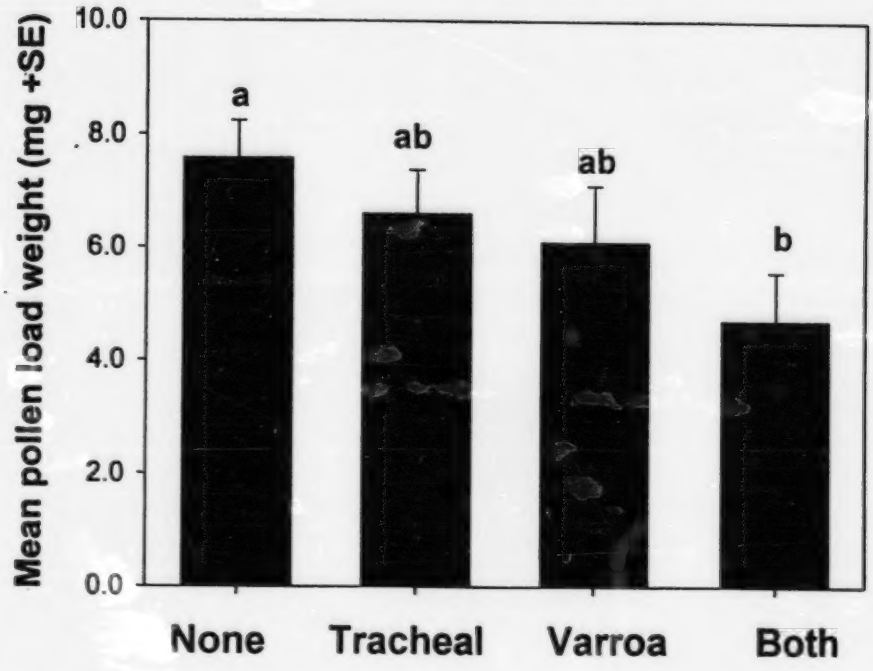

25
15
5
Mean Age (days)
a
ab
b
c
Varroa
Both
None
Tracheal


50
40
30
20
10
0
Age of foraging onset (days)
0
1
2
3
4
5
Number of Varroa in cell


Mean pollen load weight (mg +SE)
10.0
8.0
6.0
4.0
2.0
0.0
a
ab
ab
b
None
Tracheal
Varroa
Both

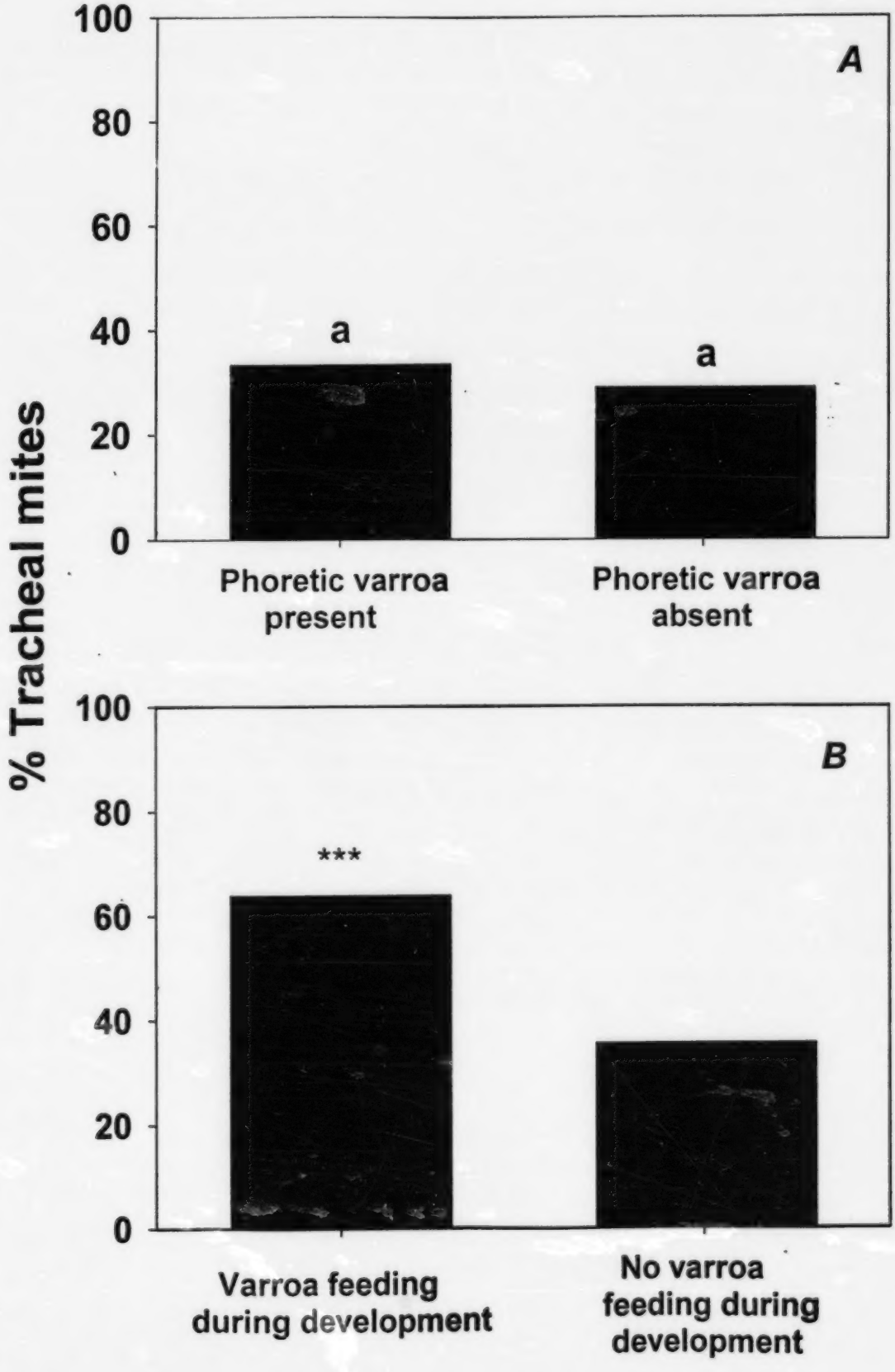

% Tracheal mites
100
80
60
40
20
0
A
a
a
Phoretic varroa present
Phoretic varroa absent
100
80
60
40
20
0
B
***
Varroa feeding during development
No varroa feeding during development

FIGURE LEGENDS

Figure 1: Effect of mite parasitism on mean age (± SE) of foraging onset. Letters indicate means that are significantly different ($p < 0.005$) using Duncan's Multiple Range test.

Figure 2: Relationship between age at foraging onset and number of varroa mites in the bee's cell during development. (Linear regression: $y = 19.55x - 1.47$, $p < 0.0001$, $r^2 = 0.0329$, $n = 991$).

Figure 3: Weight of pollen loads (mean ± SE) with tracheal and varroa mites. Means with different letters are significantly different (GLM with orthogonal contrasts, $p < 0.05$).

Figure 4A: Tracheal mite prevalence (%) in bees with and without phoretic varroa. Treatments are not significantly different ($X^2 = 0.772$, $p > 0.05$. d.f. = 1).

Figure 4B: Tracheal mite prevalence (%) in bees with and without varroa parasitism during development. Treatments are significantly different ($X^2 = 22.613$, $p < 0.001$, d.f. = 1).

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Evaluation of the Botanical Oils Neem, Thymol, and Canola Sprayed to Control *Varroa jacobsoni* Oud. (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in Colonies of Honey Bees (*Apis mellifera* L., Hymenoptera: Apidae)

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Running Head: Botanical Oils as Acaricides

Abstract

The botanical oils neem, thymol, and canola were examined for control of parasitic mites (*Varroa jacobsoni* and *Acarapis woodi)* in honey bee (*Apis mellifera*) colonies. Neem oil spray (5% solution) killed 90 ± 6% of varroa mites, three times more than died in the untreated group. Thymol-oil spray (4.8g thymol/L in 20% canola oil solution), thymol in vermiculite (3.6g/vermiculite block), and canola oil spray (20% solution) killed 79 ± 8%, 68 ± 6%, and 65 ± 6% of the varroa mites respectively. Colonies treated with the thymol-oil spray had a significantly lower tracheal mite infestation (1.3 ± 7.5%) at the end of the treatment period than the untreated group (23.3 ± 6.0%). All other treatments showed a slight but not statistically significant decrease in tracheal mite infestation levels. Neem and thymol-oil spray treatments were detrimental to bees, as they both had 50% queen loss, and colonies treated with neem oil had one third as many adult bees and one sixth as much brood as untreated colonies at the end of the experiment. However, both neem and thymol-oil spray treatments were as effective as commercially available formic acid against varroa mites. The negative effects on bees may be remedied by changes in formulation, application technology, and season of application. Neem, thymol, and canola oil treatments provide adequate control of varroa mites to be useful in an integrated pest management approach. These natural products may have the added advantage of

simultaneously reducing tracheal mite populations. Thus, continued research to develop application methods that do not cause bee mortality is warranted.

Key Words

***Apis mellifera* / *Varroa jacobsoni* / *Acarapis woodi* / acaricide / neem / thymol / canola oil**

INTRODUCTION

Varroa jacobsoni (Oudemans) is an ectoparasite of the Asian honey bee (*Apis cerana*) that has become a major pest of *Apis mellifera* in the last few decades (DeJong *et al.*, 1984; DeJong, 1990). Varroa mites were discovered in *Apis mellifera* L. colonies in Germany in 1977 and have since spread throughout Europe and North America (Koeniger and Fuchs, 1989). There are few pesticides available that adequately control varroa mites and do not leave chemical residues in honey or wax. In Canada and the United States fluvalinate (Apistan®, Wellmark International, Texas) is licensed for use as an acaricide to control varroa mite populations. Formic acid (Medivet Pharmaceuticals Ltd., Alberta) also is licensed for use in Canada, and coumaphos (Perizin®, Bayer) is currently licensed in parts of the United States under emergency use registration. In various European countries, coumaphos, amitraz (Anti-varroa®, Schering), and thymol (ApiLife VAR®, LAIF Chemicals), have been used to control varroa

mites (Imdorf *et al.*, 1995; Eischen, 1998). Although fluvalinate, coumaphos, and amitraz can have greater than 95% efficacy, varroa mites have or likely will develop resistance to them (Henderson, 1988; Herbert *et al.*, 1988a, 1988b; van Buren *et al.*, 1992; Abed and Lahitte, 1993; Lodesani *et al.*, 1995; Elzen *et al.*, 1998). Formic acid is less effective, with 70-94% varroa control (Hoppe *et al.*, 1989; Feldlaufer *et al.* 1997). To minimize or prevent resistance, new acaricides are needed, and existing acaricides should be used in rotation. Furthermore, new acaricides that do not leave residues in honey or wax that could be used during the honey flow are needed. The ideal acaricide would kill a high proportion of varroa mites without killing honey bees. It would not induce resistance in mites, leave residues in wax or honey, be toxic to other non-target organisms, and would require only one annual application.

Tracheal mites (*Acarapis woodi* Rennie) also are a significant worldwide pest of honey bees (Delfinado-Baker, 1984). They have been present in Europe for many years and were first discovered in the United States in 1984 (Delfinado-Baker, 1985). Since that time, tracheal mites have spread throughout the United States and Canada. Tracheal mites decrease the lifespan of individual honey bees (Maki *et al.*, 1986; Eischen, 1987). Heavy infestation can cause poor overwintering, a decrease in honey production, and colony death (Eischen, 1987; Furgala *et al.*, 1989; Eischen *et al.*, 1989). Tracheal mites are particularly deleterious to honey bee colonies when varroa mites also are present (Downey *et*

al., 1999). Menthol and formic acid are the only products registered for use against tracheal mites in Canada, and only menthol is registered for use in the United States (Herbert *et al.*, 1987; Cox *et al.*, 1989; Calderone and Shimanuki, 1995; Ellis and Baxendale, 1997). The effectiveness of both products is dependent on ambient temperature, as the amount of active compound released varies with temperature. Other compounds such as amitraz and cymiazole (Apitol®, Ciba-Geigy Ltd.) are effective against tracheal mites, but they have not received regulatory approval in the United States or Canada (Eischen *et al.*, 1986; Dietz *et al*, 1987; Eischen *et al.*, 1989; Hoppe *et al.*, 1989;). Vegetable oils in a sugar patty formulation have been used to prevent tracheal mites levels from increasing (Delaplane, 1992; Sammataro *et al.*, 1994; Calderone and Shimanuki, 1995).

Recently, there has been interest in the acaricidal properties of botanical oils due to their low mammalian toxicity. Neem oil (from the Asian neem tree *Azadirachta indica*), thymol (from *Thymus vulgaris L.*) and canola oil (rapeseed oil, from *Brassica napus*) are three products that we investigated. Previous experiments have shown all three oils to have activity against varroa mites (Chiesa, 1991; Calderone and Spivak, 1995; Imdorf *et al.*, 1995; Calderone *et al.*, 1997; Melathopoulos *et al.*, 1999b) and other honey bee diseases (Melathopoulos *et al.*, 1999a), thereby reducing the number of chemicals used in honey bee hives.

Commercial production of botanical oil products have not been successful because effective formulations and application technology have not been determined. In addition, formulations that are consistently effective against varroa and tracheal mites have not been found. Application technologies that release oils throughout the hive are most effective against mites, but those tested to date have a single source of release that result in uneven distribution of oils throughout the hive. Spray applications might be particularly effective at spreading oils evenly throughout the hive and delivering the botanical oils directly onto the bees. Sprays applied to the top of the hive, or at the hive entrances could be dispersed throughout the hive by bee movement.

The objectives of our experiment were to test three novel approaches to controlling varroa and tracheal mites with botanical oils: spraying oils directly onto the bees, applying neem oil as a fall treatment, and combining canola oil and thymol into one formulation. Sprays of neem oil, two doses of canola oil and thymol combined with canola oil were tested in the fall of 1998. Thymol was also applied in vermiculite blocks to compare with the spray treatments.

MATERIALS AND METHODS

Colonies

Forty-eight colonies, each in two deep Langstroth hive bodies, were used to test five botanical oil formulations. The experiment was conducted in October,

1998 in the Fraser Valley of south-western British Columbia, Canada. Pre-treatment and post-treatment adult populations and brood areas of the colonies were assessed, following the protocol outlined in Burgett and Burikam (1985), during the week before treatment applications commenced and the week following the termination of treatment applications. All colonies had high varroa mite levels (157 $\pm$ 107 mites dropped naturally per day in the week before the treatments started). The experimental colonies also had moderate tracheal mite levels (15 $\pm$ 25.9% of the bees were infested with tracheal mites). The colonies were grouped by adult population and brood area, then the colonies in each group were distributed evenly across treatments. There were six treatment groups with eight colonies in each treatment. Colonies were treated six times, once every four days. Two different colony manipulations were used in treatments, sprays and vermiculite block. Although the opening of treated colonies may have led to stress on the colonies, an undisturbed and untreated control group was used as the best comparison for the two types of colony disturbance.

Compounds

The five treatments were 5% neem spray, 20% and 50% canola oil sprays, 4.8g thymol/L in 20% canola oil spray (thymol-oil spray), and a thymol-impregnated vermiculite block (thymol block) with 3.6g thymol per block. The oils were mixed with water and 2% emulsifier (Tween 20, Sigma, St. Louis, Missouri). Water and 2% emulsifier were used as a control treatment in a

previous experiment, and were found to have no effect on either honey bee or varroa mite mortality (Melathopoulos *et al*, 1999b). The sprays were applied to the colonies using a SVV™ survivor sprayer (SP systems, California) at 63psi. Approximately 400mL of solution was sprayed onto each colony at a rate of 400mL/min.. The bottom box of bees was sprayed first, and then the top box was sprayed after being placed back onto the colony. The frames in each hive were parted, and the adult bees on the face of each frame were lightly sprayed with the solution. Neem oil was pressed seed oil from Trifolio-M (Germany), canola oil was purchased from Canada Safeway Ltd. (Calgary, Alberta), and the thymol was 99.5% pure, manufactured by Sigma (St. Louis, Missouri).

The dose of thymol used in the thymol block was the lowest dose (21.4g over 24 days) that showed efficacy against varroa in previous experiments by Calderone *et al.* (1997). Calderone *et al.* (1997) showed the thymol blocks release half of the thymol over the treatment period. Therefore, half of the dose used in the thymol blocks was used in the thymol-oil spray (10.7g over 24 days), as the thymol would be sprayed directly onto the bees. The thymol-oil spray was composed of thymol dissolved in canola oil mixed with water and emulsifier. The thymol blocks were made of 80mm x 110mm x 7mm blocks of vermiculite. Thymol crystals (3.6g) were dissolved in 38mL hexane and then the vermiculite blocks were impregnated with the thymol/hexane solution. The hexane was allowed to evaporate, leaving only thymol crystals in the vermiculite block. The

thymol blocks were placed into a wire mesh screen (3mm) to prevent bees from chewing the vermiculite block, and were replaced once every four days for twenty-four days to ensure a constant release rate over the treatment period. Thymol release rate from vermiculite blocks at 23^0C was 0.31 ± 0.01g per day (n=9).

Population Monitoring

Varroa levels were monitored before, during, and after the experiment with sticky boards (Calderone *et al.*, 1997). Sticky boards were placed into the colonies on the bottom boards for three days before the treatments commenced to determine the relative abundance of varroa mites in each of the colonies, and replaced every eight days during and after treatments. As sticky boards remove from the varroa population those mites that fall to the bottom board, the efficacy of these treatments are those found when used in conjunction with a bottom board varroa trap.

After the treatment period, the colonies were assessed for adult populations and brood areas. The day following the assessment, fluvalinate strips (Apistan®) were placed into the hives and left for forty-two days to kill remaining mites in each colony. Mite levels were monitored for three weeks after the Apistan® strips were placed in the hives.

Pre-treatment and post-treatment tracheal mite levels were determined for each colony by collecting 30 bees from the inner cover of each colony in 70%

ethanol. The bees were collected immediately before the treatments commenced, and shortly after they ended. Tracheal mite dissections were carried out as outlined by Shimanuki and Knox (1991). Tracheal mites were scored as present or absent in the trachea of dissected bees. Due to time restraints, it was not possible to carry out live tracheal mite dissections to determine if the tracheal mites observed were alive or dead. Therefore, treatments may be more effective than reported.

Analysis

The efficacy of each treatment was determined by dividing the varroa treatment mortality by the total varroa mortality (treatment drop + post-treatment drop). Varroa efficacy and tracheal mite infestation data were both proportional, and so they were transformed by root arcsine (Sokal and Rohlf, 1981). Unless otherwise stated, means of all data were compared using the Tukey-Kramer HSD multiple comparison test (JMP IN, SAS Institute Inc. 1996). Treatment effects on colony mortality and queen loss were compared using a modified Student-Neuman-Keuls procedure (Steel and Torrie, 1980; Jones, 1984).

RESULTS

Varroa Control Efficacy. There were significant differences overall in efficacy of varroa treatments ($P<0.0001$). The neem spray was significantly more effective against varroa than either the 20% canola spray or the untreated

group ($P<0.05$) (Fig. 1). All of the treatments were significantly more effective against varroa mites than the untreated group ($P<0.05$) (Fig. 1).

Tracheal Mite Control Efficacy. Tracheal mite levels between treatment groups were not different at the beginning of the experiment ($P=0.9771$), and the control of tracheal mites was not significantly different between treatments ($P=0.1942$). However, the tracheal mite infestation level was significantly lower in the thymol-oil spray group than in the untreated group at the end of the treatment period ($P<0.05$) (Fig. 2). There was a decrease in tracheal mite levels in all groups except the untreated group.

Post-treatment Colony Size. Colony sizes were not different at the beginning of the experiment with respect to the adult bee population ($P=0.9790$) or brood area ($P=0.8575$), and so the final size of treatment groups was compared to determine if there was a treatment effect on colony size. Prior to treatment, the average size ($\pm$ S.E.) of the colonies was 12,214$\pm$ 4423 adult workers and 2332.4 $\pm$ 909.0 cm^2 brood. Small colonies were used for ease of experimentation. The adult worker population of all treatment groups decreased, except for the thymol block treatment group, which remained the same (Fig. 3). The thymol block treatment had significantly more workers at the end of the treatment than all but the untreated group ($P<0.05$) (Fig. 3). The neem spray had significantly less brood than the untreated group at the end of the experiment ($P<0.05$) (Fig. 3).

The final brood area of all the treatment groups was lower than the pretreatment brood area (Fig. 3).

Colony and Queen Loss. The number of queens lost in the neem and thymol-oil spray treatments were significantly higher than in the untreated group ($P<0.05$) (Fig. 4). No colonies or queens were lost in either the thymol block group or the untreated group. All colonies in the 50% canola spray group died by the end of the third treatment application.

DISCUSSION

The botanical oils and formulations tested here demonstrated 60-90% efficacy against varroa mites and diminished tracheal mite infestations, but were detrimental to the bees. The neem and thymol-oil spray treatments induced the most varroa mite death, and the thymol-oil spray showed the greatest reduction in tracheal mite levels. However, these treatments also resulted in reduced colony populations and queen loss. The 20% canola spray and the thymol block had similar varroa and tracheal mite control, while the 50% canola oil spray had the greatest colony mortality. Thymol-oil spray had slightly higher mite death than the thymol block, but the thymol block was superior to the thymol-oil spray as a treatment since it did not kill as many bees.

These results indicate potential for using neem and thymol as miticides, but the impact of botanical oil treatments on bees needs to be decreased

by modifying the application technology or changing the season of application. This experiment was conducted in the fall when temperatures were cool, and some bee mortality may have resulted from the bees being wet from the sprays in cool air temperatures. Summer spray treatments were not as injurious to bees and showed little measurable impact (Melathopolous *et al.*, 1999b). Decreasing the total amount of liquid sprayed while maintaining the same level of active ingredient also could reduce bee mortality.

Although neem oil caused bee mortality, it is a promising acaricide as it is toxic to a wide variety of mites while it has low toxicity to many beneficial insects (Schmutterer, 1988). Neem oil can reduce varroa and tracheal mite levels in honey bee colonies when applied in spring and summer (Melathopoulos *et al.*, 1999b). Neem also has inhibitory affects on bacteria, and so it is possible that it will inhibit the growth of bacterial honey bee pathogens such as American foulbrood (Rao *et al.*, 1986; Williams *et al.*, 1998).

Thymol has been presented in a vermiculite block as a control agent for varroa and tracheal mites with varying success (Calderone and Spivak, 1995; Imdorf *et al.*, 1995; Calderone *et al.*, 1997). Thymol is the main active ingredient in ApiLife VAR®, which has been used for varroa control with 92-99% efficacy in Europe (Imdorf *et al.*, 1994). However, similar formulations with thymol as the main active ingredient and the same application methods produced 50-97% varroa mortality (Calderone and Spivak, 1995; Calderone *et al.*, 1997). As is true

for many botanical oils, the lethal concentrations for bees and varroa mites are close (Lindberg *et al.*, 1999), and thymol applications can injure bee colonies (Chiesa, 1991). The effects of thymol on bees and mites depend considerably on temperature, as temperature affects the amount of thymol sublimation. Thymol residues left in honey bee colonies treated with ApiLife VAR® are not significant, are well below the taste threshold, and are not dangerous to the consumer (Imdorf *et al.*, 1995).

Canola oil and other vegetable oils are used in patty formulations to maintain tracheal mites at low levels (Calderone and Shimanuki, 1995). Preliminary experiments in which canola oil was sprayed onto bees in concentrations of 10% and 20% provided significant reduction in varroa mite populations (Melathopoulos *et al.*, 1999b). Although high concentrations of canola oil cause bee mortality, there is potential to reduce both varroa and tracheal mite populations simultaneously with canola oil sprays.

Sprays may not be the best method of applying oils because of potential effects on bees, but our results suggest that methods which better distribute oils through the hive may improve the efficacy of oils over single-source applications. Wicking devices that release oils by bee contact, foggers that spray oil droplets in the entrance or the top of the hive, and entrance blocks that release oils at the hive entrance with returning bees distributing the oils throughout the nest are delivery

options that should be evaluated. We are pursuing research into these types of delivery systems for botanical oils.

Our results indicate that neem or thymol-oil spray have potential for mite control. Neem is particularly interesting, since in previous experiments sprayed formulations of neem applied during the spring or summer controlled varroa mites and did not harm the bees (Melathopoulos *et al.*, 1999b). Applications of neem or thymol-oil spray are as effective or more effective than formic acid. It seems likely that continued work with these substances will lead to viable application methods.

Botanical oils alone or in combination, including neem, canola, and thymol, have potential as controls for varroa mites. Botanical oils will not eliminate varroa or tracheal mites, but they may be able to reduce levels of these honey bee pests when used with other methods in an integrated pest management system. Many botanical oils are recognized as food-grade substances, significantly reducing the cost of registration. Formulation and application technology need to be improved before these products can be licensed for use as safe, reliable controls for varroa and tracheal mites. Also, long-term studies to determine the level of varroa and tracheal mite control required to reduce injury to acceptable levels are needed to better evaluate miticides for use in an integrated pest management system. We encourage the research community to focus on these areas.

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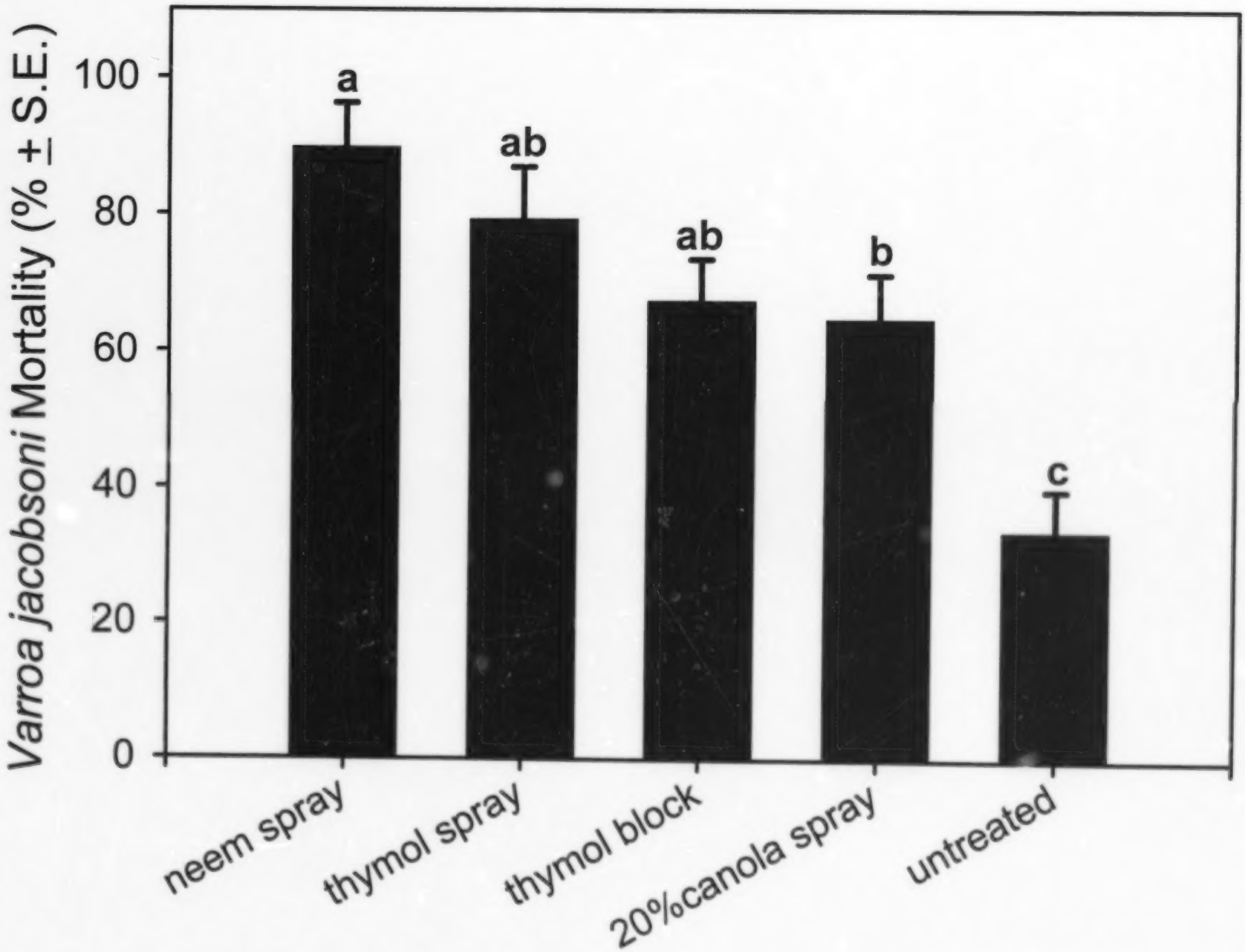
Figure Legends

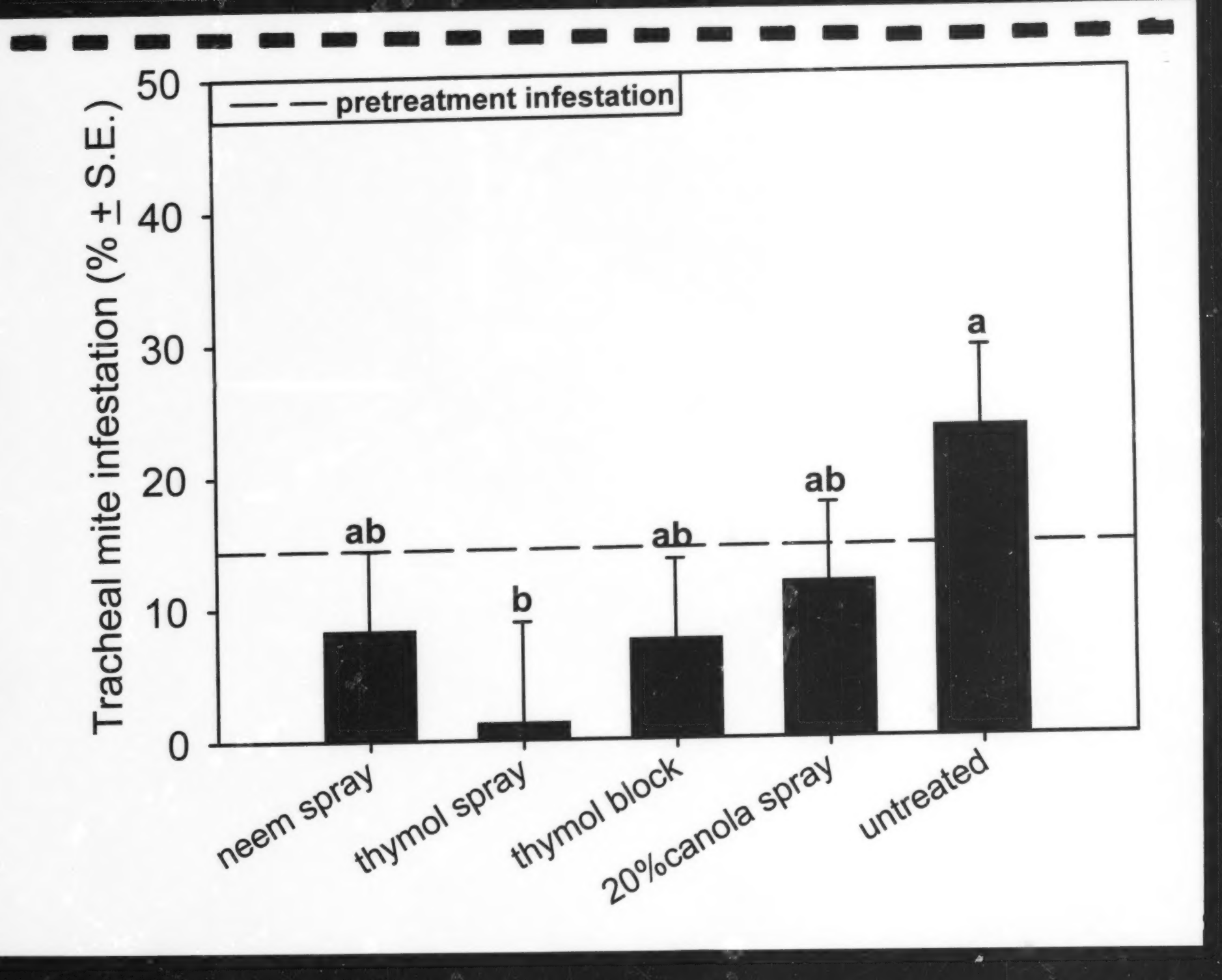
Fig. 1. The efficacy of each treatment against *Varroa jacobsoni*. Means with the same letters were not significantly different (Tukey's $P<0.05$).

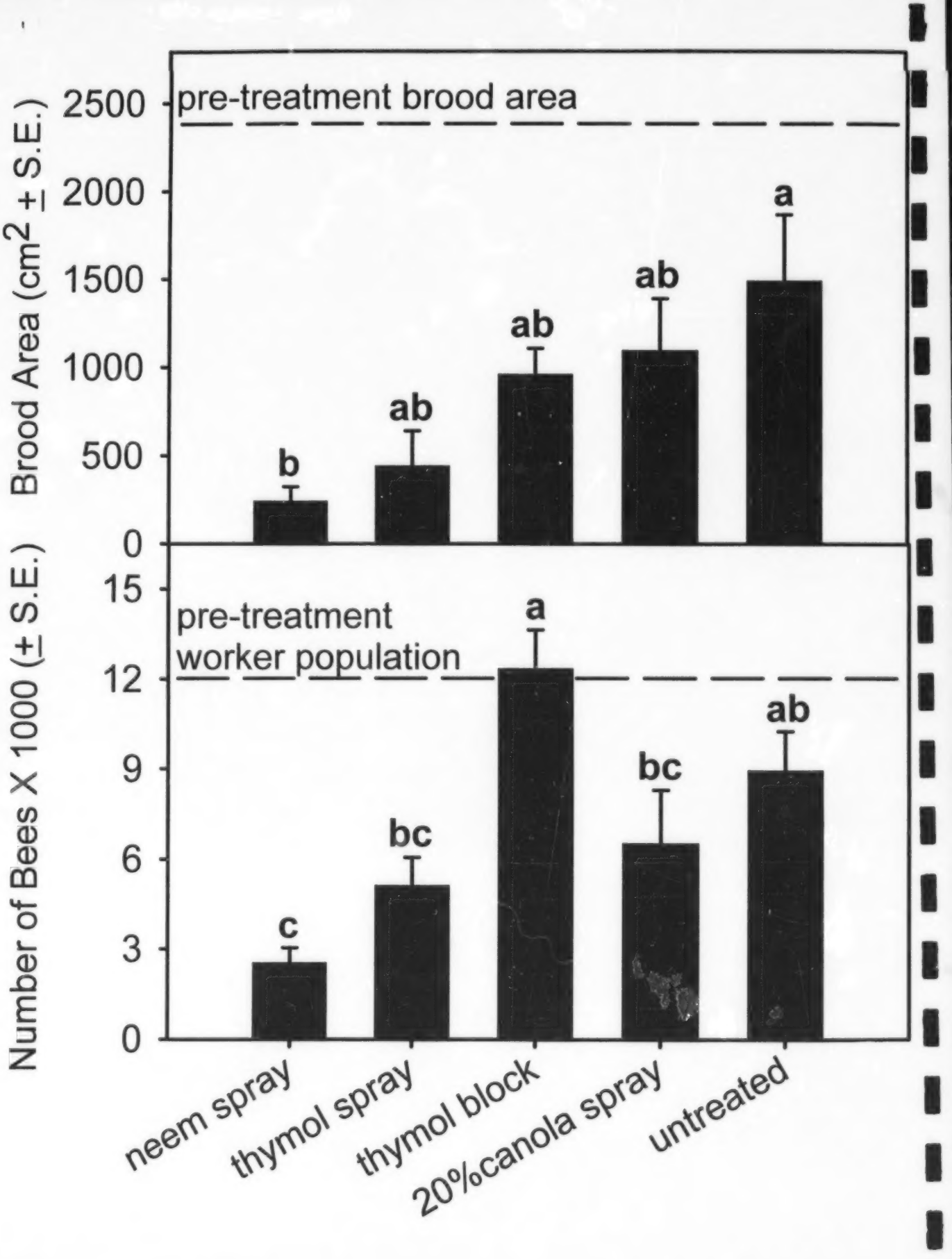
Fig. 2. The effects of treatment on tracheal mite infestations. Means with the same letters were not significantly different (Tukey's $P<0.05$).

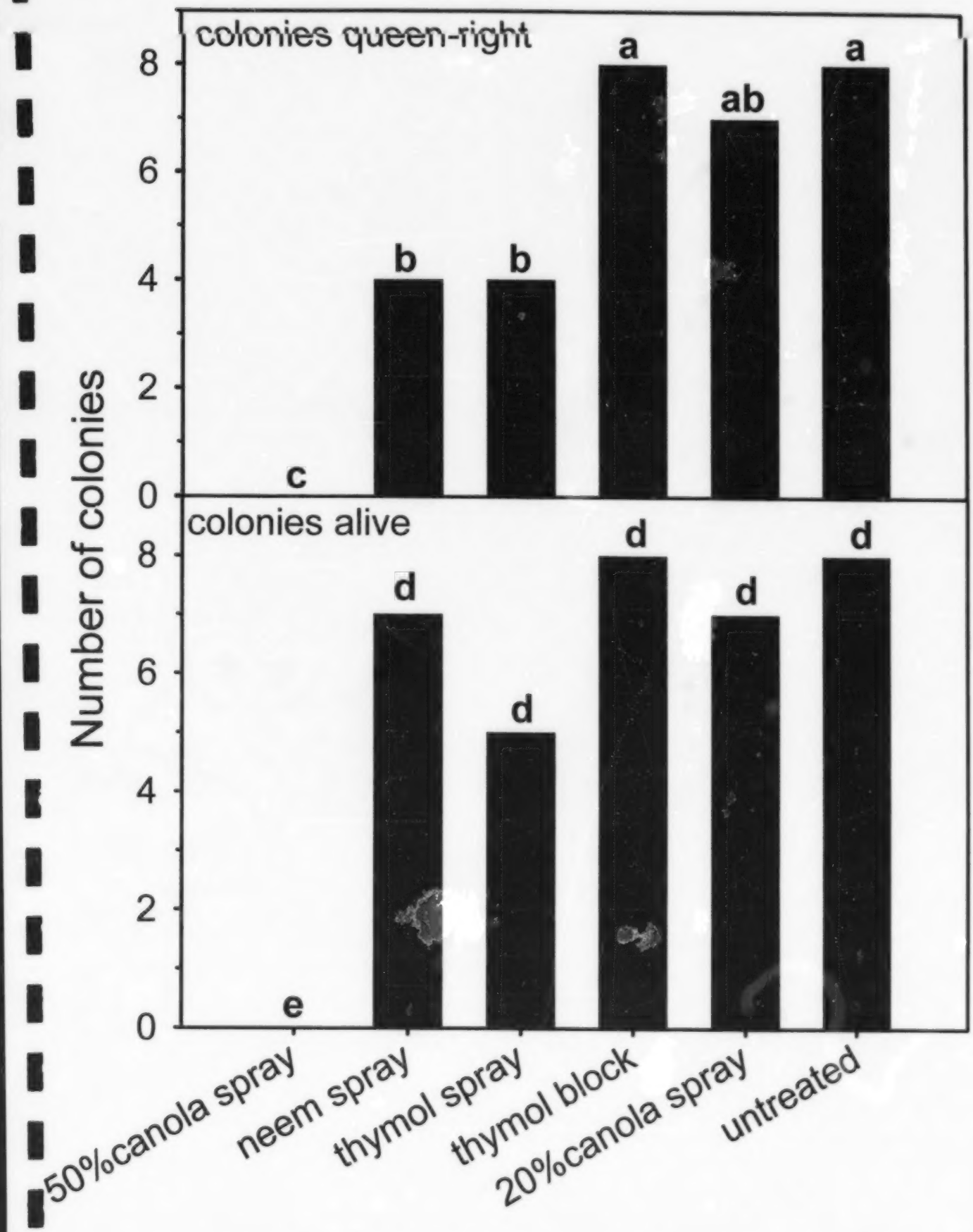
Fig. 3. Worker population and brood area of treatment groups at the end of the treatment period, compared to pretreatment worker population and brood area. Means with the same letters were not significantly different (Tukey's $P<0.05$).

Fig. 4. The number of colonies alive and queen-right at the end of the treatment period. Means with the same letters were not significantly different. (modified Student-Neuman-Keuls).


Varroa jacobsoni Mortality (% ± S.E.)
100
80
60
40
20
0
a
ab
ab
b
c
neem spray
thymol spray
thymol block
20%canola spray
untreated


pretreatment infestation
Tracheal mite infestation (% ± S.E.)
50
40
30
20
10
0
ab
b
ab
ab
a
neem spray
thymol spray
thymol block
20%canola spray
untreated


pre-treatment brood area
2500
2000
1500
1000
500
0
b
ab
ab
ab
a
Brood Area (cm2 ± S.E.)
pre-treatment
worker population
15
12
9
6
3
0
c
bc
a
bc
ab
Number of Bees X 1000 (± S.E.)
neem spray
thymol spray
thymol block
20%canola spray
untreated


colonies queen-right
colonies alive
Number of colonies
0
2
4
6
8
a
ab
a
b
b
c
d
d
d
d
d
e
50%canola spray
neem spray
thymol spray
thymol block
20%canola spray
untreated